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ISOLATION OF AN ENDOGENOUS GROWTH REGULATOR FROM AGRICULTURAL CROPS
АУЫЛ ШАРУАШЫЛЫҚ ДАҚЫЛДАРЫНАН ЭНДОГЕНДІ ӨСУ РЕТТЕУІШІН БӨЛІП АЛУ

ANNOTATION

This article explores the use of biological products with growth-stimulating and immunomodulatory effects in agricultural practice, focusing on the production and influence of growth regulators on grain crops, specifically the high-grade winter wheat variety Steklovidnaya-24 from KazNIIZiR. The study aims to develop an endogenous growth regulator and assess its impact on grain crops. Phytohormones, as endogenous growth regulators, play a crucial role in plant growth and development, controlling various physiological and morphogenetic programs. The research involves obtaining growth regulators from cereal crops, with a particular focus on the cytokinin N6-benzylaminopurine (6-BAP). The experiments, conducted under hydroponic conditions, reveal the positive influence of growth regulators on seed germination and biometric indicators, such as increased linear dimensions and root system volume. The results demonstrate that the 1% concentrated solution of wheat extract with 6-BAP exhibited the highest germination rate, showcasing its potential as an effective growth regulator for winter wheat seeds. Overall, the study emphasizes the importance of natural growth regulators for sustainable agricultural practices and highlights the specific impact of cytokinins on seed germination and plant growth.

Key words: *Phytohormone, 6-BAP, auxin, gibberellin, cytokinin, ethylene, photosynthesis.*

Introduction. In recent years, considerable attention has been devoted to the development of plant protection techniques in agriculture, emphasizing the utilization of biological products with growth-stimulating and immunomodulatory effects. A diverse range of bioregulators has been formulated for major crops, reflecting the ongoing efforts to enhance agricultural practices.

The modification of proteins through ubiquitination is crucial for both human health and the regulation of plant growth, development, and their responses to different environmental stresses [1-5].

Ubiquitin-mediated protein stability significantly influences plant growth and development. The ubiquitin-proteasome system (UPS) functions in both the cytoplasm and nucleus, controlling protein levels and removing misfolded or damaged proteins that may accumulate due to abiotic stress [6]. The necessity for employing immunomodulators in agricultural crops stems from their inherent challenges, including uneven seed germination, an extended period from sowing to emergence, and slow initial growth during the growing season. Such vulnerabilities make these plants more susceptible to pest damage and diseases. It has been established that pre-sowing treatment of medicinal crop seeds with growth regulators stimulates both germination energy and the overall germination process.

Growth regulators play a pivotal role in the cultivation of various crops. While artificially synthesized growth regulators like auxin, gibberin, and cytokinin exhibit similar effects, the pursuit of natural growth regulators remains crucial for obtaining organic stimulants without adverse environmental effects. Therefore, research focused on acquiring natural regulators holds contemporary relevance. Auxin, mainly in the form of indole-3-acetic acid (IAA), is a crucial phytohormone that governs plant growth and development. IAA influences plant morphogenesis, organ development, aptitude response, and tissue differentiation throughout a plant's growth cycle. In fruit tree cultivation, IAA promotes cutting rooting, enhances stress tolerance, and regulates secondary metabolism in fruits [7-11].

Endogenous growth regulators, synthesized by the plant itself, include well-known phytohormones. These chemical substances regulate various aspects of plant growth and development at low concentrations, interacting with receptors on the cell surface, leading to changes in cellular processes. Phytohormones serve as the primary factors for regulation and control in plants, influencing entire physiological and morphogenetic programs.

Phytohormones activate key ontogenetic programs, particularly in seed germination, marked by the exit from dormancy and rapid accumulation of vegetative mass [13]. Water absorption, triggered by physical and osmotic processes, initiates germination, progressing through phases like seed swelling, pecking, heterotrophic growth of seedlings, and the transition to an autotrophic mode of nutrition [14-16].

Researchers acknowledge hormones as pivotal factors in seed germination, emphasizing their concentration-specific effects. Notably, the plant growth hormone N6-benzylaminopurine (6-BAP), related to cytokinin, plays a significant role in seed germination and development. This work aims to study the influence of growth regulators on grain crop growth, involving tasks such as obtaining an endogenous growth regulator from cereal crops and examining their impact on overall growth [17-19].

During seed germination, phytohormones enter the cotyledons from the axial parts of the embryo and influence the development of the activity of enzymes involved in the breakdown of reserve substances. The resulting decomposition products enter the axial parts of the embryo and ensure their growth. Isolated pumpkin cotyledons are highly sensitive to exogenous phytohormones, since after isolation they rapidly deplete the supply of endogenous hormones. In the works of M.K. Gilmanov showed that exogenous cytokinin significantly activates the growth of cotyledons. In this case, the influence of cytokinin extends to all aspects of the formation of intracellular structures and the metabolism of cotyledons [20-21].

In accordance with the activation of the growth of cotyledons and the processes of intracellular differentiation associated with their transformation into a green leaf, cytokinins trigger in the cotyledons the synthesis of a variety of enzymes necessary for the deployment of these physiological programs.

The purpose of this work is to study the influence of growth regulators on the growth of grain crops. To achieve this goal, the following tasks were set:

- Obtain an endogenous growth regulator from cereal crops;
- To study the impact of regulators on the growth of grain crops.

Material and methods. Growth regulators were obtained from the highest grade of grain seeds of agricultural crops. The object of the study was grain agricultural crops of KAZNIIZiR winter wheat of the highest grade Arai Steklovidnaya-24. The experiments were carried out in the laboratory under hydroponic conditions. Previously, the seeds were sterilized with sodium hypochlorite (NaOCl) 2% for 10 minutes, washed to pH = 7. For the appearance of endogenous growth regulators, the seeds were immersed in a solution containing 1% 6-benzylaminopurine (Sigma Aldrich, Germany). Seeds without 6-BAP were used as a control sample.

Hydroponics conditions were created for the germination of grain crop seeds. Plant seedlings were grown in a hydropol installation at a temperature of 24±20 C, humidity 60 and 16 hours of light per day at 1500 lux.

The experiment was carried out as follows:

Seed sterilization → Germination → Separation of seedlings from the embryo → Homogenization → Centrifugation → Separation of the supernatant → Obtaining a growth regulator.

Solutions of 0.5%, 1%, 2% and 100% concentration were prepared from two samples.

For each sample, 5 Petri dishes were prepared. 20 Root of premium wheat seeds Arai Steklovidnaya-24 were poured inside.

The first day was watered with distilled water, 20 ml for each Petri dish.

On the second day, the wheat began to sprout (stem and root).

On the third day, the prepared solutions with two samples were poured:

1.Wheat extract

2. Wheat extract treated with 6-BAP.

Growth regulators obtained from extracts from grain plants were studied for the germination of winter wheat grain seeds.

Research results and their discussion. Seed germination was assessed visually by counting seedlings (Table 1). Results are presented as percentages. Biometric features were studied by measuring plants.

Table 1 shows the effect of plant extracts obtained from grain agricultural crops on the germination of winter wheat seeds. 4 samples of different concentrations of wheat extract and a control sample in water were studied. On the first day of planting, they watered it with 20 ml of distilled water; on the second day they did not water it because there was enough moisture in the Petri dish. On the second day, shoots appeared in the seeds, the root size was 0.9 ± 1 cm and the stem length was 3 ± 4 mm. On the third day, they watered with a concentrated solution of 20 ml.

After watering with a concentrated solution, growth regulators showed the following results after 1 day:

Sample No. 1 - (control sample) - stem of seedlings 0.3 cm, root -1.1 cm;

Sample No. 2 - (0.5% concentrated solution) - stem - 0.5 cm, root - 1.3 cm;

Sample No. 3 - (1% concentrated solution) stem - 0.3 cm, root - 0.6 cm;

Sample No. 4 - (2% concentrated solution) stem - 0.6 cm, root - 1.3 cm

On the sixth day, all samples were watered again. It was found that in sample No. 1 the stem is 8.5 cm, the root is 5.7 cm; Sample No. 2 stem – 7 cm, root – 2.7 cm; Sample No. 3 stem – 7.2 cm, root – 4 cm, Sample No. 4 stem – 4 cm, root – 3.3

On the ninth day, all samples were watered again with 10 ml. The following results were obtained: sample No. 1 stem – 17.8 cm, root – 14 cm; Sample No. 2 stem – 17 cm, root – 8 cm; Sample No. 3 stem – 13.5 cm, root – 5.5 cm; Sample No. 4 stem – 9.1 cm, root – 4.5 cm.

It has been shown that sample No. 1 has the highest germination rate.

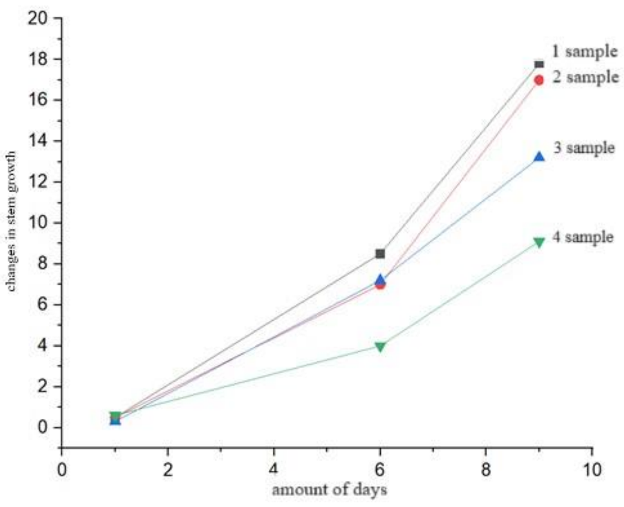

Figure 1 – Changing stem growth using a growth regulator without 6-BAP

Table 1 – Study of the effect on the germination of winter wheat Steklovidnaya-24 using wheat extract

№	Sample No. 1 - Control on water			Concentrated extract 100%			Sample No. 2 -0.5% solution			Sample No. 3 - 1% solution			Sample No. 4 - 2% solution		
	date	**watering**	Sprout size and quantities	date	watering	Sprout size and quantities	date	watering	Sprout size and quantities	date	watering	Sprout size and quantities	date	Watering	**Sprout size and quantities**
	Number of seeds - 20 pieces														
Day 1	18.10.23	20 ml	Sprouts-11 pcs Stem-0,3 cm Root-1,1 cm	18.10.23	-	-	18.10.23	20 ml	Sprouts-16 pcs Stem-0,5 cm Root-1,3 cm	18.10.23	20 ml	Sprouts-13 pcs Stem-0,3 cm Root-0,6 cm	18.10.23	20 ml	Sprouts-18 pcs Stem-0,6 cm Root -1,3 cm
Day 6	23.10.23	20 ml	Sprouts-20 pcs Stem-8,5cm Root-5,7 cm				23.10.23	20 ml	Sprouts – 20 pcs Stem-7 cm Root-2,7cm	23.10.23	20 ml	Sprouts – 20 pcs Stem-7,2 cm Root-4 cm	23.10.23	20 ml	Sprouts – 20 pcs Stem-4 cm Root-3,3 cm
Day 9	26.10.23	10 ml	Sprouts-20 pcs Stem-17,8 cm Root-14 cm				26.10.2023	10 ml	Sprouts-20 pcs Stem-17 cm Root-8 cm	26.10.2023	10 ml	Sprouts-20 pcs Stem-13,5 cm Root-5,5 cm	26.10.2023	10 ml	Sprouts-20 pcs Stem-9,1 cm Root-4,5 cm

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Table 2 – Study of the effect on the germination of winter wheat Steklovidnaya-24 using wheat extract treated with 6-BAP

№	Sample No. 1 - Control on water			Concentrated extract 100%			0.5% solution			1% solution			2% solution		
	date	watering	Sprout size and quantities	date	watering	Sprout size and quantities	date	watering	Sprout size and quantities	date	watering	Sprout size and quantities	date	watering	Sprout size and quantities
Day 1	18.10.23	20 ml	Sprouts-8 pcs Stem-0,3 cm Root-0,9cm	18.10.23	20 ml-H_2O	Nothing appeared, nothing grew	18.10.23	ml- $_2O$	Sprouts-14 pcs Stem -0,5 мм Root-0,7 cm	18.10.23	20 ml-H_2O	Sprouts-12 pcs Stem-0,7 cm Root – 1,7 cm	18.10.23	20 ml-H_2O	Sprouts-9 pcs Stem-0,7 cm Root-1,4 cm
Day 6	23.10.23	20 ml	Sprouts-20 pcs Stem-8,5 cm Root-3,8 cm	23.10.23	-		23.10.23	20 ml	Sprouts-20 pcs Stem-8 cm Root- 4 cm	23.10.23	20 ml	Sprouts-20 pcs Stem-11,6 cm Root-6,5 cm	23.10.23	20 ml	Sprouts-20 pcs Stem-7,6 cm Root- 6 cm
Day 9	26.10.23	10 ml	Sprouts-20 pcs Stem-13,5 cm Root-5,5 cm				26.10.23	10 ml	Sprouts-20 pcs Stem-8,5 cm Root-5,3 cm	26.10.23	10 ml	Sprouts-20 pcs Stem-18,5 cm Root – 9,3 cm	26.10.23	10 ml	Sprouts-20 pcs Stem-15,3 cm Root-5,5 cm

The influence of extracts on the germination of winter wheat seeds 0.5% concentrated wheat extract on the second day the percentage was 55%, on the third day 80% and on the fourth day 100%. 1% concentrated wheat extract germination percentage on the second day is 35%, on the third day 65% and on the fourth day 100%. 2% concentrated wheat extract percentage on the second day is 85% and the third day is 100%. At 100% conc. There was no germination in the solution.

In table No. 2, the germination effect of extracts obtained from grain agricultural crops and the germination of winter wheat seeds. For research, we took 4 samples of different concentrations of wheat extract with the addition of 6-BAP and a control. The first day of planting was watered with 20 ml of distilled water; on the second day it was not watered because there was sufficient moisture in the Petri dishes. Shoots appeared in the seeds, the root size was 0.9 ± 1 cm and the stem length was 3 ± 4 mm. The third day they watered the first time with a concentrated solution. After watering with a concentrated solution, growth regulators showed the following results after 1 day:

Sample No. 1 - (control variant) stem – 0.3 cm, root – 0.9 cm;

Sample No. 2 - 0.5% (concentrated solution) stem – 0.5 cm, root – 0.7 cm;

Sample No. 3 - 1% (concentrated solution) stem – 0.7 cm, root – 1.7 cm;

Sample No. 4 - 2% (concentrated solution) stem – 0.7 cm, root – 1.4 cm, grew more.

On the sixth day, all samples were watered again. It was revealed that in Sample No. 1 the stem is 8.5 cm, the root is 3.8 cm; Sample No. 2 stem – 8 cm, root – 4 cm; Sample No. 3 stem – 11.6 cm, root – 6.5 cm; Sample No. 2 stem – 7.6 cm, root – 6 cm.

On the ninth day, all samples were watered again with 10 ml. The following results were obtained: sample No. 1 stem -13.5 cm, root -5.5 cm; Sample No. 2 stem – 8.5 cm, root – 5.3 cm; Sample No. 3 stem – 18.5 cm, root – 9.3 cm; Sample No. 4 stem – 15.3 cm, root – 5.5 cm.

According to the results, the highest growth of stem and root was shown by a 1% concentrated solution of wheat extract with 6-BAP.

Table 3 – Percentage germination rate of winter wheat seeds

№	Amount of days	Control, % germination	0.5% conc. solution, % germination	1% conc. solution, % germination	2% conc. solution, % germination	100% solution, % germination
1	II day	40 %	70 %	60 %	45%	0%
2	III day	50 %	100 %	100 %	100%	0%
3	IV day	100%				0%

The effect of extracts on the germination of winter wheat seeds was 0.5% concentrated wheat extract with 6-BAP. On the second day the percentage was 40%, on the third day 50% and on the fourth day 100%. 1% concentrated wheat extract germination percentage on the second day is 70%, on the third day 100%. 2% concentrated wheat extract percentage on the second day is 45% and the third day is 100%. There was no germination in the 100% concentrated solution.

Conclusion. Cytokinin (6-BAP) increases the activity of endopeptidase in cotyledons, which is involved in the hydrolysis of proteins, alkaline and acid pyrophosphatases, which destroy pyrophosphate formed during the synthesis of various biopolymers, and thereby contribute to the occurrence of these syntheses. Cytokinin activates the malik enzyme, which is involved in the metabolism of organic acids, and ribulosebisphosphate carboxylase, a key enzyme in photosynthesis. Enzymes, according to the degree of their activation by cytokinin, can be divided into two groups: one, in its response to the phytohormone, correlates with the growth of cotyledons, the other - with the accumulation of chlorophyll in them. Enzymes of the first group are more closely related to the growth program, the second - with the biochemical differentiation of chloroplasts. Cytokinin stimulates protein synthesis in cells, acting at different levels of this process. The hormone activates RNA synthesis, increases the number of ribosomes in the cell, and increases their activity in protein synthesis.

In our work, we obtained an endogenous growth regulator from agricultural crops of the highest grade winter wheat, Arai Steklovidnaya-24. From the results obtained it follows that under the influence of 6-BAP the protein-enzyme composition is catalyzed and the biosynthesis of plant growth regulators occurs.

As a result of the study on germination and the influence of regulators on seed growth, the highest result was shown by wheat extract with 6-BAP.

The data presented in tables 1, 2 allow us to judge the positive impact of the studied plant growth regulators on seed germination and biometric indicators: the linear dimensions of plants and the volume of the root system increase. Shoots from seeds treated with Rostock and Epin-extra appeared earlier than the control by 7 -10 days, Kemiro-Hydro – for 4 days. The best percentage of seed germination was found when treated with Rostock 99.26% compared to the control 75.7%.

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ТҮЙІН

Бұл мақалада астық дақылдарына, атап айтқанда, Стекловидная-24 КазНИИЗиР жоғары сортты күздік бидай сортына өсу реттегірсерінің өндірісі мен әсеріне ерекше назар аудара отырып, ауыл шаруашылығы тәжірибесінде ынталандырушы және иммуномодуляциялық әсерлері бар биологиялық өнімдерді қолдану талқыланады. Зерттеудің мақсаты – эндогендік өсу реттегішін жасау және оның дәнді дақылдарға әсерін бағалау. Фитогормондар эндогендік өсу реттегірсері ретінде өсімдіктердің өсуі мен дамуында маңызды рөл атқарады, әртүрлі физиологиялық және морфогенетикалық бағдарламаларды бақылайды. Зерттеудің мақсаты - цитокинин N6-бензиламинопуринге (6-БАП) ерекше назар аудара отырып, дәнді дақылдардан өсу реттегірсерін алу. Гидропоникалық жағдайда жүргізілген тәжірибелер өсу реттегірсерінің тұқымның өнуіне және тамыр жүйесінің сызықтық өлшемдері мен көлемінің ұлғаюы сияқты биометриялық көрсеткірсерге оң әсерін көрсетті. Нәтижелер бидай сығындысының 6-БАП бар 1% концентрлі ерітіндісі күздік бидай тұқымдарының тиімді өсу реттегіші ретіндегі потенциалын көрсете отырып, ең жоғары өну жылдамдығын көрсетті. Жалпы, зерттеу тұрақты ауылшаруашылық тәжірибелері үшін табиғи өсу реттегірсерінің маңыздылығын көрсетеді және цитокининдердің тұқымның өнуі мен өсімдіктердің өсуіне ерекше әсерін көрсетеді.

РЕЗЮМЕ

В данной статье рассмотрено использование биопрепаратов со стимулирующим и иммуномодулирующим действием в сельскохозяйственной практике, уделено особое внимание производству и влиянию регуляторов роста на зерновые культуры, в частности на сорт озимой пшеницы высокого сорта Стекловидная-24 КазНИИЗиР. Целью исследования является разработка эндогенного регулятора роста и оценка его влияния на зерновые культуры. Фитогормоны как эндогенные регуляторы роста играют важнейшую роль в росте и развитии растений, контролируя различные физиологические и морфогенетические программы. Целью исследования является получение регуляторов роста из зерновых культур с особым акцентом на цитокинин N6-бензиламинопурин (6-БАП). Эксперименты, проведенные в условиях гидропоники, выявили положительное влияние регуляторов роста на всхожесть семян и биометрические показатели, такие как увеличение линейных размеров и объема корневой системы. Результаты показывают, что 1%-ный концентрированный раствор экстракта пшеницы с 6-БАП показал самую высокую скорость прорастания, демонстрируя его потенциал в качестве эффективного регулятора роста семян озимой пшеницы. В целом, исследование подчеркивает важность естественных регуляторов роста для устойчивой сельскохозяйственной практики и подчеркивает специфическое влияние цитокининов на прорастание семян и рост растений.

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